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Drosophila Dalmatian combines sororin and shugoshin roles in establishment and protection of cohesion

Takashi Yamada, Eri Tahara, Mai Kanke, Keiko Kuwata and Tomoko Nishiyama

Corresponding author: Tomoko Nishiyama, Nagoya University

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1st Editorial Decision 19 September 2016

Thank you for submitting your manuscript on sister chromatid cohesion protection in Drosophila by dalmatian to our editorial office. We have now received the reports of three three expert referees, copied below for your information. Since all referees are very positive about this work and its quality, we shall be happy to consider this manuscript further for publication in The EMBO Journal. Nevertheless, the reviewers raise a number of specific concerns and questions that would need to be satisfactorily addressed before publication. I am therefore returning the manuscript to you with an invitation to revise it and to respond to the various individual points.

In line with a comment of referee 2, please also re-consider the manuscript's title and abstract to better emphasize the key finding of combined shugoshin/sororin roles in this fly homolog.

REFEREE REPORTS

Referee #1:

This interesting manuscript describes the role of the Drosophila Dalmatian (Dmt1) gene in the establishment and maintenance of cohesion in cultured cells. The results provide evidence that Dmt1 performs the functions of both sororin (previously thought to be vertebrate-specific) and shugoshin. Regions of interaction for PP2A and HP1 are identified and used to demonstrate the importance of these interactions for cohesion. The discovery that sororin and shugoshin-like functions are combined in a single protein in Drosophila will advance our evolutionary understanding of cohesion establishment and maintenance and should be of broad interest. On the whole, the study combines a logical set of experiments to provide a convincing argument. A few exceptions are outlined below:

1. The cohesion assay is very subjective and could be prone to artefacts as the ability to see separated chromosomes could be simply due to how well spread the chromosomes are. This should be supported by FISH to show the separation of sister chromatids at one or more specific regions. This has been performed for interphase cells but it would be very valuable to perform this experiment in mitosis. This is the case throughout the manuscript. Furthermore, using FISH at different regions would establish whether Dmt1 cohesion function is restricted to the pericentromeric region or not.
2. Related to the distinction between possible roles of Dmt1 at the pericentromere and the rest of the chromosomes, the authors refer to a ChIP-seq experiment in the discussion. Inclusion of this data would greatly strengthen the manuscript.
3. Figure 1B. More details of the scoring methods are needed. What does the "undetermined" category (a large fraction of total cells scored) represent?
4. Figure 1E needs a loading control to show similar amounts of extract loaded in all cases (particularly important for the double depletion).
5. Figure S1. The error bars are very large. Is the difference between wild type and cdh1 RNAi statistically significant?
6. Mass spectrometry results showing interactions with Dmt1 should be shown in full.
7. Figure 6. How did the authors identify the PP2A-binding region of Dmt1? Was this through homology or experimental analysis? These details should be provided. How do they explain their observations that PP2A, but apparently not direct binding to Dmt1, is required for Dmt1 localization?
8. Figure S7C. The image shown comparing Mei-S322 and Dmt1 localization in mitosis is not very clear and a better image could be shown.
9. The data in Figure S7D is not definitive. In many organisms, shugoshin mutants only show defects in biorientation following challenges. The authors could test this by monitoring recovery following treatment with microtubule-depolymerising drugs or after mild depletion of kinetochore proteins. Criteria to measure chromosome segregation should also be reported.
10. In vertebrate cells, sororin localization depends on acetylated Smc3. Does Dmt1 require Deco to associate with heterochromatin/cohesin?
11. Is Dmt1 enriched in the heterochromatin solely through its interaction with HP1 or is it under similar controls to shugoshin? It would be interesting to test the dependence of Dmt1 localization on Bub1 kinase.
12. D. willistoni: The Dmt1 homolog still appears to associate with foci close to heterochromatin. Could it be that a different targeting motif is responsible for targeting to heterochromatin in this organism? It is perhaps premature to suggest that the lack of N terminal domain is responsible for fragile chromosomes in this organism.
13. The manuscript would be strengthened by confirming the role of Dmt1 in cohesion in flies. Are double Dmt1 Wpl1 mutants viable?
14. Please leave a white gap between different micrograph channels, this is absent in some figures.

Referee #2:

This manuscript describes a functional analysis of Drosophila melanogaster Dalmatian (Dmt), a homolog of mammalian Sororin, which is required for the establishment of sister chromatid cohesion. Intriguingly, Dmt localizes at heterochromatin in interphase through an interaction with HP1, and this interaction is required for the establishment of cohesion. In addition to the establishment of cohesion, the authors demonstrate that Dmt is required for the maintenance of cohesion in mitosis, which is usually mediated by shugoshin proteins in mammalian cells. The authors analysed various mutant Dmt proteins defective in their association with specific proteins, and performed many solid experiments. I strongly recommend this manuscript for the publication in EMBO Journal. Addressing the points listed below would improve the manuscript.

Comments:
1) This study strikingly demonstrates that the Drosophila sororin homolog Dmt takes over the shugoshin role in mitosis, providing an evolutional insight into cohesion protection mechanisms. This could be highlighted in the abstract or even in the title.

2) The data demonstrate that Dmt localizes at heterochromatin in interphase through its interaction with HP1 proteins, and cohesin stabilizes its binding to chromatin. However, whether HP1 is

required for the Dmt localization in mitosis is unclear. In the case of mammalian cells, the interphase Sgo1 localization at heterochromatin is not required for the centromeric localization in mitosis (Perera et al., JCS 2010), but Sgo1-HP1 association supports the stable binding of Sgo1 at chromatin (Tanno et al, Science 2015). The requirement of HP1 and cohesin for Dmt localization in mitosis should be shown. It is formally possible that cohesin is required for the localization of Dmt during mitosis (Fig. 2D). The requirement of PP2A for the localization of Dmt also could be explained as a consequence of the cohesin reduction. It would be nice to see the metaphase localization of Dmt mutants defective in cohesin- or HP1-binding.

3) Fig. 1A: The definition of 'partially separated' is not clear from the representative pictures.

4) Fig. 1A: Please show representative images of mis-segregation.

5) Fig. 1D: Please show representative image of FISH staining.

6) Fig. 3F: How about the localization of the full-length Dmt-VEIE mutant in interphase and in mitosis? How about the contribution of HP1 and cohesin to the mitotic localization of Dmt? Please see comment 1.

7) Fig. S4: This figure is not required for the manuscript. It is unclear whether the cohesion defect in DwDmt-expressing S2 is indeed due to the failure of heterochromatin localization. It is not clear whether DwDmt has a or not role in cohesion.

8) Figure 5C: How about the cohesin interaction in Dmt-ΔCPB pull-down?

9) Figure 5H: How do the authors classify the 'stable' and 'dynamic' fractions of Dmt-GFP in FRAP analysis? This could be included in the method section.

10) Please show a list of the interacted proteins identified by the mass spectrometry analysis.

11) Figure 6B: 87B-mCherry (PP1) also seems to co-localize with Dmt. Some quantification is required.

12) It is not shown how the PP2A binding region (PPB) and cohesin-Pds5 binding domain (CPB) were identified in Dmt.

13) The authors show that the centromeric localizations of Dalmatian and PP2A are interdependent. However, Dmt-ΔPPB, which cannot bind to PP2A, could localize normally to centromeric heterochromatin (Fig S6B). This requires some explanation.

14) Fig7A: The authors state that Sgo1-GFP accumulates on pericentromeric heterochromatin. However, the signals are very faint and obscure in human cells.

15) Does the expression of Dmt-ΔPPB suppress Sgo1 RNAi in human cells?

Referee #3:

This interesting manuscript, by Yamada and co-workers, provides evidence that Dalmatian-Dmt, an orthologue of human Sororin (cohesin establishment factor) is also a functional equivalent to human Shugoshin (involved in the protection of centromeric cohesion to the prophase pathway during mitosis). They further demonstrate that Dmt localization to heterochromatic regions is cohesin-independent but relies on HP1 binding and this interaction is required for cohesion. The most interesting aspect of the paper is the report that, similarly to Shugoshin, Dmt recruits PP2A, and is unable to protect cohesin without PP2A. In line of the functional similarity between human Shugoshin and Drosophila Dmt, Dmt is able to rescue sister chromatid cohesion in Shugoshin mutated mammalian cells.

These are novel and exciting new findings that bring two important contributions to the field: 1) it provides a unique example of protein function overlap across evolution 2) it solves the "mystery" of

cohesion protection in the fly, which for long has been quite puzzling. I am therefore highly favourable towards this manuscript. There are, however, several experimental issues that should be addressed before I can fully recommend it for publication.

Comments:
1. Throughout Figure 1 (and in several other figure in the paper) the authors should perform statistical analysis on their results to support their conclusions and probably increase the number of independent experiments. Particularly as the results are not consistent throughout the different panels (note the different degrees of sister chromatid cohesion scored for controls and Dmt RNAi), which points to strong variability across experiments. Whereas for Fig. 1A it is clearly stated n=3 for all the others it is only stated the number of cells. Are these from a single independent experiment?
2. In a large number of the experiments the authors claim that a construct rescues/does not rescue is made in a very strong manner, although careful inspection of the graphs demonstrates more subtle differences (e.g. "Dmt-depleted cells was significantly suppressed by depletion of Wapl (p5)" This is also particularly true for data on Figure 4A) The text should be modified to better match the data.
3. The localization of Dmt presented in figure 2 is very convincing and the microscopy stunning. However, the finding that cohesin does not follow the same localization, as previously described in neuroblasts, is quite puzzling. Could the authors speculate on why these differences may occurs?
4. In figure 3A the authors show that upon Dmt RNAi, Scc1 is still present on chromatin, which is quite surprising considering published results and the authors own data. Are the levels the same? What about the levels of smc3 measured in fixed samples (as in Fig. 3B)?
5. The authors convincingly demonstrate that Dmt binds to heterochromatin in a HP1-dependent manner. However, the claims regarding the importance of this interaction for cohesion should be toned down. In Figure 4 authors claim that WT Dmt rescues although looking at the graph it is clear that the rescue is only to about 50% and the mutants also rescue to about 30%. Although the tendency is there, the effects are rather mild (again, statistics could support the claims).
6. The results regarding PP2A interaction/localization are probably the strongest points of the paper. Although the rationale for the use of WAPL depletion is clear, Figure 6C should nevertheless include quantifications of the respective controls (wild-type cells) to clarify if the bimodal behavior of wdb-GFP localization if also present in otherwise unperturbed cells, or a consequence of WAPl depletion (maybe WAPL itself also interacts with PP2A).
7. For the FRAP data on figure 5GH, did the authors control for cell cycle stage of the cells analyzed? This should be important as cohesion stability if known to change significantly upon replication.
8. The rescue experiments presented in Figure 7 are indeed quite remarkable and a very convincing argument for the major claims of the manuscript. It would nevertheless be important to control that the levels of all constructs are equivalent (either WB or quantifications of GFP levels).

Minor points:

9. The sentence "RNA interference (RNAi) of Dmt resulted in defective cohesion in the control cells (p5)" is confusing and should be rephrased.
10. Figure 1C needs a legend;
11. The authors should included further details on how the data in figure 1D was scored? Are they measuring only G2 cells? How are they identified?
12. Figure 1E lacks loading control (is the decrease in scc1 levels upon Dmt+Wapl RNAi consistent?)
13. Figures 5F-G should include a label that it refers to Dmt-GFP

In summary, this manuscript reports very exciting findings. Some of the conclusions need to be further supported, as the experimental set-up (multiple RNAi/rescue experiments) is prone to intrinsic experimental variability. However, if all the concerns are addressed and the conclusions are well documented this manuscript should be of prime interest for EMBO Journal readers.

1st Revision - authors' response 05 March 2017

Reply to the Referees' Comments

We would like to thank all the referees for their positive comments on our manuscript and for providing many helpful suggestions. We would like to answer the referee's comments point by point. Our responses are shown in italics.

Referee #1:

This interesting manuscript describes the role of the Drosophila Dalmatian (Dmt1) gene in the establishment and maintenance of cohesion in cultured cells. The results provide evidence that Dmt1 performs the functions of both sororin (previously thought to be vertebrate-specific) and shugoshin. Regions of interaction for PP2A and HP1 are identified and used to demonstrate the importance of these interactions for cohesion. The discovery that sororin and shugoshin-like functions are combined in a single protein in Drosophila will advance our evolutionary understanding of cohesion establishment and maintenance and should be of broad interest. On the whole, the study combines a logical set of experiments to provide a convincing argument. A few exceptions are outlined below:

1. The cohesion assay is very subjective and could be prone to artefacts as the ability to see separated chromosomes could be simply due to how well spread the chromosomes are. This should be supported by FISH to show the separation of sister chromatids at one or more specific regions. This has been performed for interphase cells but it would be very valuable to perform this experiment in mitosis. This is the case throughout the manuscript. Furthermore, using FISH at different regions would establish whether Dmt1 cohesion function is restricted to the pericentromeric region or not.

I would like to thank the referee for this suggestion. According to the suggestion, we established FISH method for analyzing mitotic cohesion by using pericentromeric ChX probe. We now changed all chromosome spread data, except Fig 5A, to corresponding new FISH results. Because we needed to evaluate "over cohesion" phenotype, which was indistinguishable from normal cohesion state in pericentromere FISH, we left chromosome spread data in Fig 5A. Instead, we explained the definition of "separated" category in the legend in Fig 5A.

Regarding probes for different regions, we could not establish the FISH approach for chromosome arm regions because of their faint staining on mitotic chromosomes. Nevertheless, we believe that Dmt can establish cohesion on euchromatic region on chromosome arms because 1) interphase arm FISH experiments indicated the distances between 2 sisters in euchromatic

region was decreased by expression of Dmt, 2) over-cohered mitotic chromosome arms were observed after Dmt overexpression, and 3) ChIP-seq analysis (new Appendix Figure S7) showed that Dmt is accumulated in chromosome arm region. We mentioned it in Discussion (p.17-18).

2. Related to the distinction between possible roles of Dmt1 at the pericentromere and the rest of the chromosomes, the authors refer to a ChIP-seq experiment in the discussion. Inclusion of this data would greatly strengthen the manuscript.

We added the ChIP-seq data as representative snap shots in Appendix Figure S7. This ChIP-seq data support the assumption that Dmt is colocalized not only with HP1 but also with cohesin on euchromatin.

3. Figure 1B. More details of the scoring methods are needed. What does the "undetermined" category (a large fraction of total cells scored) represent?

As the referee pointed out, "not determined" cells were abundant and the difference between "not determined" and "mis-segregation" was less clear in the previous version. We now repeated the time-lapse imaging and changed the classification. In the new figure, cells treated with control or Dmt dsRNA were stained by Hoechst 33342 (for DNA) and sirTub (for microtubule) after MG132 treatment to enrich metaphase cells. In those living cells, chromosome alignment was evaluated and classified into "normal (aligned)" and "scattered (misaligned)". This result is now shown in new Figure 1B.

4. Figure 1E needs a loading control to show similar amounts of extract loaded in all cases (particularly important for the double depletion).

As requested, we added tubulin blot as a loading control in new Figure 1G.

5. Figure S1. The error bars are very large. Is the difference between wild type and cdh1 RNAi statistically significant?

As the referee pointed out, the Dmt-GFP intensities in Cdh1-depleted cells were varied especially in later time points after cell division because Dmt-GFP was greatly accumulated in

some Cdh1 RNAi cells. We now plotted all the actual values in 200-min time window so that the difference between control and Cdh1 RNAi cells become clearer. It is now shown in Figure EV1B.

6. Mass spectrometry results showing interactions with Dmt1 should be shown in full.

As requested, Mass Spectrometry result is shown in new Table S2.

7. Figure 6. How did the authors identify the PP2A-binding region of Dmt1? Was this through homology or experimental analysis? These details should be provided. How do they explain their observations that PP2A, but apparently not direct binding to Dmt1, is required for Dmt1 localization?

Dmt^{PPB} (275-299) were identified based on Dmt-Wdb binding assay with various truncations of Dmt. We added this process in new Appendix Figure S5F. Because the binding amount of Wdb to Dmt was significantly decreased in Dmt-ΔN299 compared to Dmt-ΔN274, we assume that $Dmt^{275\text{-}299}$ is required for association with Wdb. This result does not necessarily predict that $Dmt^{275\text{-}299}$ is directly bound to Wdb, but $Dmt^{275\text{-}299}$ could be required for "indirect" binding between Dmt and PP2A.

Regarding the requirement of PP2A for Dmt localization, a major cause of Dmt mislocalization in Wdb + B′ RNAi cells was sister separation. Because more than 40 % of the Wdb + B′ RNAi cells were defective in cohesion (Appendix Figure S5B), we speculate that this mainly caused Dmt mislocalization. However, we revealed that even in the cells with apparent normal cohesion, the Dmt signals were sometimes significantly diminished in Wdb + B′ RNAi cells (Figure 6D). When we measured Dmt intensities only in cohered chromosomes in each condition, they were significantly decreased in Wdb + B′ RNAi cells (new Figure 6E), indicating that PP2A-B′ could directly or indirectly affect association of Dmt on peri-centromeric heterochromatin. Although it remains unknown if sister separation is result from Dmt dissociation or, oppositely, mild separation causes Dmt dissociation in Wdb + B′ RNAi cells, we speculate that 1) PP2A-B′ changes phosphorylation state of Dmt, which could be important for stable chromatin- or cohesin-binding to Dmt as in the case of Sororin (Nishiyama et al., 2013), or 2) pericentromere structure is somehow ensured by PP2A. We mentioned this in the main text (p.12-13).

8. Figure S7C. The image shown comparing Mei-S322 and Dmt1 localization in mitosis is not very clear and a better image could be shown.

We repeated the cytospin/immunostaining experiment with anti-Dmt antibodies, which gives better images than Dmt-GFP staining. We show the pictures in new Figure EV4B.

9. The data in Figure S7D is not definitive. In many organisms, shugoshin mutants only show defects in biorientation following challenges. The authors could test this by monitoring recovery following treatment with microtubule-depolymerising drugs or after mild depletion of kinetochore proteins. Criteria to measure chromosome segregation should also be reported.

This is an important point. To test if spindle biorientation is defective in Mei-S332-depleted cells, we performed time-lapse imaging after Colcemid washout. In S2 cells, cell-cycle-release after Colcemid treatment does not work if the Colcemid concentration was high enough to depolymerize all the microtubules. Therefore, we had to treat the cells with low concentration of Colcemid. As a result of mild Colcemid treatment, very few cells were arrested in mitosis and, in most of the cases, cells entered into mitosis during time-lapse imaging. The result indicates that both the time required for Colcemid release and duration of mitosis (from NEBD to anaphase onset) were not significantly different between control- and Mei-S332 RNAi cells in S2 cells. We added this result in new Figure EV4D.

10. In vertebrate cells, sororin localization depends on acetylated Smc3. Does Dmt1 require Deco to associate with heterochromatin/cohesin?

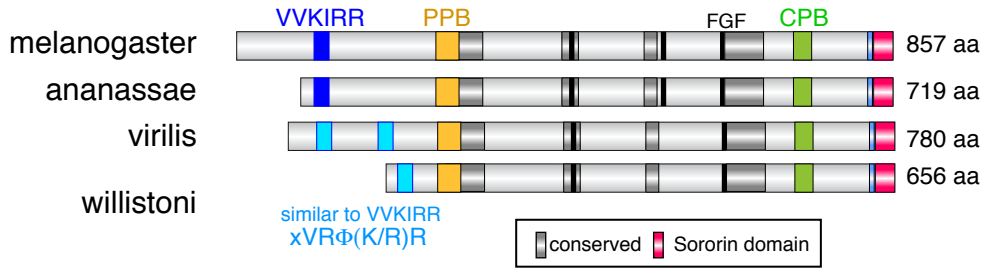
In interphase cells, San+Deco RNAi did not apparently change the localization of Dmt on heterochromatin. We added this result in new Appendix Figure S2B. Regarding cohesin binding, we performed pull-down experiment and it was revealed that Deco RNAi reduced Dmt binding to Scc1 to the similar extent to Pds5 RNAi, indicating that Dmt associates with cohesin in a similar manner to sororin. We added this result to new Appendix Figure S4A.

11. Is Dmt1 enriched in the heterochromatin solely through its interaction with HP1 or is it under similar controls to shugoshin? It would be interesting to test the dependence of Dmt1 localization on Bub1 kinase.

This is an important point to clarify the similarity to Sgo1. According to the suggestion, we tested Dmt localization after Bub1 RNAi. Bub1 RNAi enriched the cells with precocious sister separation and anaphase, therefore Dmt was hardly detected on such separated chromosomes, but otherwise cohered chromosomes exhibited normal localization of Dmt in mitosis (new Appendix Figure S6A). Generally in S2 cells, because effect of RNAi becomes apparent asynchronously, a certain population does not show the phenotype. Therefore we, so far, could not distinguish the following two possibilities; 1) Dmt was dissociated from separated chromatids because of the absence of cohesin/cohesion after Bub1 RNAi, 2) Bub1 (H2A phosphorylation) was required for pericentromeric localization of Dmt and the Dmt mislocalization caused sister separation and precocious anaphase. We need further investigation to clarify this point. We mentioned it in the main text (p.14).

12. D. willistoni: The Dmt1 homolog still appears to associate with foci close to heterochromatin. Could it be that a different targeting motif is responsible for targeting to heterochromatin in this organism? It is perhaps premature to suggest that the lack of N terminal domain is responsible for fragile chromosomes in this organism.

As the referee pointed out, D.willistoni Dmt still has a very similar motif "xVRΦ(K/R)R" in its N-terminus (highlighted in light blue in the scheme below), which is also seen in D.virilis. This second motif may contribute to associate with heterochromatin in S2 cells. Since this figure is not essential for the manuscript (and also the Referee #2 pointed it out), we decided to leave this figure out from the revised manuscript.



13. The manuscript would be strengthened by confirming the role of Dmt1 in cohesion in flies. Are double Dmt1 Wpl1 mutants viable?

We appreciate the suggestion. However, we are currently unable to keep flies in our lab. and to test the viability. Although it is definitely interesting experiment, we believe that lacking of this experiment does not harm the quality of this manuscript and change our conclusion.

14. Please leave a white gap between different micrograph channels, this is absent in some figures.

We now checked all the figures and improved the picture alignment and white gaps.

Referee #2:

This manuscript describes a functional analysis of Drosophila melanogaster Dalmatian (Dmt), a homolog of mammalian Sororin, which is required for the establishment of sister chromatid cohesion. Intriguingly, Dmt localizes at heterochromatin in interphase through an interaction with HP1, and this interaction is required for the establishment of cohesion. In addition to the establishment of cohesion, the authors demonstrate that Dmt is required for the maintenance of cohesion in mitosis, which is usually mediated by shugoshin proteins in mammalian cells. The authors analysed various mutant Dmt proteins defective in their association with specific proteins, and performed many solid experiments. I strongly recommend this manuscript for the publication in EMBO Journal. Addressing the points listed below would improve the manuscript.

Comments:
1) This study strikingly demonstrates that the Drosophila sororin homolog Dmt takes over the shugoshin role in mitosis, providing an evolutional insight into cohesion protection mechanisms. This could be highlighted in the abstract or even in the title.

As the referee suggested, we added one sentence regarding evolutional insight in the abstract, like "This provides a clue to elucidate an evolutional transition of the cohesion system in eukaryote." Because of the limit of title length and also to avoid over statement, we only mildly changed the title like "Dual role for Drosophila Dalmatian in establishment and protection of sister chromatid cohesion."

2) The data demonstrate that Dmt localizes at heterochromatin in interphase through its interaction with HP1 proteins, and cohesin stabilizes its binding to chromatin. However, whether HP1 is required for the Dmt localization in mitosis is unclear. In the case of mammalian cells, the interphase Sgo1 localization at heterochromatin is not required for the centromeric localization in mitosis (Perera et al., JCS 2010), but Sgo1-HP1 association supports the stable binding of Sgo1 at chromatin (Tanno et al, Science 2015). The requirement of HP1 and cohesin for Dmt localization in mitosis should be shown. It is formally possible that cohesin is required

for the localization of Dmt during mitosis (Fig. 2D). The requirement of PP2A for the localization of Dmt also could be explained as a consequence of the cohesin reduction. It would be nice to see the metaphase localization of Dmt mutants defective in cohesin- or HP1-binding.

As the referee pointed out, endogenous Dmt has never been detected on the separated chromosomes in any kinds of RNAi (new Figure EV2 and also Figures 2D, 3E, and Appendix Figure S6A), indicating that cohesin/cohesion is essential for mitotic localization of Dmt. Nevertheless, we tested HP1 requirement for Dmt mitotic localization. HP1a/b-depleted cells showed only mild cohesion defect, if any, in S2 cells (Appendix Figure S2D), and Dmt was hardly detectable again on such separated chromosomes. This could be explained by requirement of cohesin/cohesion. However, even in the majority of cohered chromosomes, HP1a/b RNAi significantly decreased the amount of Dmt on pericentromeres (new Figure 3F). We measured longitudinal length of Dmt-localizing area and the Dmt intensities only in cohered chromosomes and revealed that Dmt-positive area became shorter and amount of Dmt was also decreased in HP1a/b RNAi cells. We added these results to new Figures 3E and 3F.

There are two possibilities to explain this result; 1) Reduction of Dmt on mitotic pericentromere is result of its failure in interphase localization on heterochromatin; or 2) HP1 facilitate the "excess" accumulation of Dmt on pericentromeres in mitosis. In either case, HP1a/b RNAi did not completely remove Dmt from mitotic chromosomes, resulting in a very mild cohesion defect. Because Dmt^{VEIE} showed severer cohesion defect (Figure 4A) than in HP1a/b RNAi cells, it might be important to consider the contribution of other heterochromatin protein family proteins having CSD. Although these problems are still unsolved, we could at least conclude that HP1 is required for the efficient accumulation of Dmt on pericentromeric heterochromatin in mitotic chromosomes. We now mentioned it in the main text (p.8 and p.19).

3) Fig. 1A: The definition of 'partially separated' is not clear from the representative pictures.

As also suggested by the referee #1, since the chromosome spread classification was subjective, we now established FISH method for analyzing mitotic cohesion by using pericentromeric ChX probe. In the FISH experiment, we simply counted the numbers of FISH signals, which are corresponding to cohesion states (2 indicates "cohered" and 4 indicates "separated"). We now changed all chromosome spread data (except Fig 5A to evaluate over cohesion) to corresponding new FISH results.

4) Fig. 1A: Please show representative images of mis-segregation.

Since the definitions of "mis-segregation" and "not-determined" were less clear in the previous manuscript, we now repeated the live cell imaging and changed the classification. In the new experiment, cells treated with control or Dmt dsRNA were stained by Hoechst 33342 for DNA and sirTub for microtubule after MG132 treatment to enrich metaphase cells. In those living cells, chromosome alignment was evaluated and classified into "normal (aligned)" and "scattered (misaligned)". This result is shown in new Figure 1B with representative images.

5) Fig. 1D: Please show representative image of FISH staining.

As requested, we added their representative images of FISH in new Figure 1D. Note that their differences in sister distances are rather difficult to be recognized in the pictures, because the size of S2 cells is small and even cohesin knockdown does not alter the sister distance evidently.

6) Fig. 3F: How about the localization of the full-length Dmt-VEIE mutant in interphase and in mitosis? How about the contribution of HP1 and cohesin to the mitotic localization of Dmt? Please see comment 1.

We tested the localization of full length Dmt^{VEIE} ($Dmt\text{-}full^{VEIE}$-GFP) in the presence of endogenous Dmt. As shown in new Appendix Figure S3A, we unexpectedly found that both $Dmt\text{-}full^{WT}$ and $Dmt\text{-}full^{VEIE}$ exhibited normal pericentromeric localization in mitosis. Also in interphase, $Dmt\text{-}full^{VEIE}$ localized to heterochromatin (please see Figure for Referee 1 attached below). There are two possibilities to explain this result; 1) $Dmt\text{-}full^{VEIE}$ could still associate with heterochromatin though other regions of Dmt. This is conceivable because we have identified the 86-116 region through making truncations of Dmt (Appendix Figure S2F). Although VEIE mutation was sufficient to abolish heterochromatin localization of 86-116 fragment, this could be insufficient in full length.

Another possibility is 2) Dmt could form homodimer or multimer with endogenous Dmt. When we performed pull-down experiment of Dmt^{WT}-mCherry and Dmt^{WT}-GFP, Dmt^{WT}-mCherry was precipitated with Dmt^{WT}-GFP, indicating that Dmt could form multimer directly or indirectly in S2 cells (new Appendix Figure S3B). Therefore, we speculate that Dmt^{VEIE} could bind to endogenous Dmt on heterochromatin. We further speculate that the dimerization/oligomerization domain may be present in the most N-terminal region of Dmt

because Dmt-ΔN116- or Dmt-86-116(VEIE)-C320-GFP failed to localize to heterochromatin even in the presence of endogenous Dmt (Figure 3I and Appendix Figure S2F).

Regarding the contribution of HP1, please see our response to the point 2).

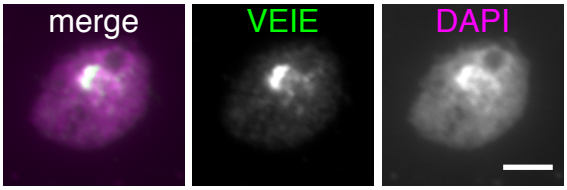


Figure for Referee 1

Dmt^{VEIE}-GFP was expressed in S2 cells and the interphase localization was observed. DNA was counter stained with DAPI. Bar: 5 μm.

7) Fig. S4: This figure is not required for the manuscript. It is unclear whether the cohesion defect in DwDmt-expressing S2 is indeed due to the failure of heterochromatin localization. It is not clear whether DwDmt has a or not role in cohesion.

Although it is clear that D.willistoni Dmt is not functional in D.melanogaster S2 cells in terms of sister chromatid cohesion, this does not necessarily predict that D.willistoni Dmt is not functional in D.willistoni cells. Nevertheless, as the referee pointed out, further investigation is needed to answer the question why D.willistoni Dmt is not functional in S2 cells. We left this figure out from the revised manuscript.

8) Figure 5C: How about the cohesin interaction in Dmt-ΔCPB pull-down?

We performed Dmt ΔCPB-GFP pull-down experiment. Dmt-WT could associate with endogenous Scc1 but the binding of ΔCPB with Scc1 was significantly reduced compared with WT, indicating that Dmt associates with cohesin through Pds5 and the binding requires CPB domain. We now show this result in new Appendix Figure S4D.

9) Figure 5H: How do the authors classify the 'stable' and 'dynamic' fractions of Dmt-GFP in FRAP analysis? This could be included in the method section.

We now explained about calculation of residence time in the method section. Briefly, because almost all Dmt proteins are chromatin-bound (Figure 2A), we assumed that there are two fractions of Dmt, namely dynamic chromatin-bound fraction and stable chromatin-bound faction. Therefore we used two-phase association curve fitting (I = $Span^{fast}$(1-exp(-k_{Off}^{fast})*time) +*

$Span^{slow}$(1-exp(-k_{Off}^{slow})*time)), where "fast" and "slow" correspond to "dynamic" and "stable" fractions, respectively. Each residence time (τ) was calculated as $\tau = 1/k_{Off}$.*

10) Please show a list of the interacted proteins identified by the mass spectrometry analysis.

We now show the mass spectrometry result in a new Table S2.

11) Figure 6B: 87B-mCherry (PP1) also seems to co-localize with Dmt. Some quantification is required.

We re-examined the localization of 87B and compared it with endogenous Dmt by immunostaining (new Appendix Figure S5A). We found that 87B and Dmt seemed to be colocalized on centromeres in some of mitotic cells but in most of the cases they exhibited the distinct localization, namely 87B was localized to kinetochore rather than centromere, which is consistent with previous reports (Bloecher and Tatchell 2000; Trinkle- Mulcahy et al. 2003). Therefore we presume that the localization of 87B cannot be distinguished from Dmt in initial stage of mitosis but its kinetochore localization become evident during metaphase.

12) It is not shown how the PP2A binding region (PPB) and cohesin-Pds5 binding domain (CPB) were identified in Dmt.

For both PPB and CPB, we identified them by pull-down experiments with various truncations of Dmt. We added the western blots showing these processes to new Appendix Figure S4B and S4C (CPB) and Appendix Figure S5F (PPB).

13) The authors show that the centromeric localizations of Dalmatian and PP2A are interdependent. However, Dmt-ΔPPB, which cannot bind to PP2A, could localize normally to centromeric heterochromatin (Fig S6B). This requires some explanation.

As the referee pointed out, we showed that the localizations of Dmt and PP2A-B' were interdependent. This interdependency is primarily based on cohesion, namely both Dmt and Wdb require cohesin/cohesion for their proper localizations (Figure 6C and EV2). However, even in the apparently cohered chromosomes, Dmt and Wdb could affect the localization of each other

(new Figure 6E and Appendix Figure S5C-E). It is still unclear if sister separation in Wdb + B' RNAi cells is result from Dmt dissociation or, oppositely, mild separation causes Dmt dissociation. Nevertheless, we assume the following possibilities; 1) PP2A-B' RNAi alters phosphorylation state of Dmt, which could dissociate Dmt from mitotic chromosomes as in the case of Sororin (Nishiyama et al., 2013), or 2) pericentromeric structure is somehow disordered by PP2A- B' RNAi. We mentioned it in the main text (p.12-13).

Regarding Dmt-ΔPPB localization, there are two possibilities to explaining why the localization of ΔPPB was normal (Appendix Figure S5H); 1) Wdb-Dmt interaction through PPB is not essential for Dmt localization although it's essential for cohesion protection. In this case, PP2A-B' may ensure Dmt localization through its catalytic activity rather than the interaction through PPB. However, 2) we cannot not rule out the possibility that the ΔPPB localization was not properly evaluated in our assay because of the presence of endogenous Dmt. We showed in the revised manuscript that Dmt could form dimer/multimer directly or indirectly (new Appendix Figure S3B). It is possible that endogenous Dmt tether Dmt-ΔPPB to pericentromeres. We speculate that the dimerization/multimerization domain may be present in the most N-terminal region (1-85aa) of Dmt because neither Dmt-ΔN116- nor 86-116^{VEIE}-C320-GFP localized to heterochromatin in the presence of endogenous Dmt (Figure 3I and Appendix Figure S2F). We mentioned this in the main text (p.13).

14) Fig7A: The authors state that Sgo1-GFP accumulates on pericentromeric heterochromatin. However, the signals are very faint and obscure in human cells.

In the previous figure, we took an unfocused Z section from the original confocal images. We apologize the mistake. Now we selected a focused section from the same confocal image.

15) Does the expression of Dmt-ΔPPB suppress Sgo1 RNAi in human cells?

This is an important experiment that we did not perform. We now performed the Sgo1 rescue experiment by Dmt ΔPPB and ΔCPB in RPE-1 cells. As shown in new Figure 7E and 7F, both ΔPPB and ΔCPB could partially restore cohesion in Sgo1 RNAi cells. Interestingly, cohesion defect in ΔCPB was milder than in ΔPPB cells, implying that deficiency of Dmt in cohesin (Pds5) binding (ΔCPB) only mildly affected the protection activity in Sgo1 depleted human cells, presumably because cohesion has been established by endogenous Sororin. On the other hand, ΔPPB was less active compared with WT, implying the conserved protection system presumably using PP2A should exist.

Referee #3:

This interesting manuscript, by Yamada and co-workers, provides evidence that Dalmatian-Dmt, an orthologue of human Sororin (cohesin establishment factor) is also a functional equivalent to human Shugoshin (involved in the protection of centromeric cohesion to the prophase pathway during mitosis). They further demonstrate that Dmt localization to heterochromatic regions is cohesin-independent but relies on HP1 binding and this interaction is required for cohesion. The most interesting aspect of the paper is the report that, similarly to Shugoshin, Dmt recruits PP2A, and is unable to protect cohesin without PP2A. In line of the functional similarity between human Shugoshin and Drosophila Dmt, Dmt is able to rescue sister chromatid cohesion in Shugoshin mutated mammalian cells.

These are novel and exciting new findings that bring two important contributions to the field: 1) it provides a unique example of protein function overlap across evolution 2) it solves the "mystery" of cohesion protection in the fly, which for long has been quite puzzling. I am therefore highly favourable towards this manuscript. There are, however, several experimental issues that should be addressed before I can fully recommend it for publication.

Comments:

1. Throughout Figure 1 (and in several other figure in the paper) the authors should perform statistical analysis on their results to support their conclusions and probably increase the number of independent experiments. Particularly as the results are not consistent throughout the different panels (note the different degrees of sister chromatid cohesion scored for controls and Dmt RNAi), which points to strong variability across experiments. Whereas for Fig. 1A it is clearly stated n=3 for all the others it is only stated the number of cells. Are these from a single independent experiment?

In revised manuscript, we performed mitosis FISH experiments instead of chromosome spreads. In FISH, we could evaluate mitotic cohesion by simply counting the numbers of FISH signals, which is more reliable way than classification like "cohered" or "partially cohered" in a previous manuscript. In all mitotic FISH experiments, we performed 3 experiments (n = 3) and more than 20 cells were observed in each condition in each experiment. We changed all the result of chromosome spread, except Figure 5A, to FISH and mention the statistics in their legends. Regarding Figure 5A, because we needed to evaluate "over cohesion" phenotype, which is indistinguishable from normal cohesion in pericentromere FISH, we left chromosome spread data in Figure 5A as previous version.

2. In a large number of the experiments the authors claim that a construct rescues/does not rescue is made in a very strong manner, although careful inspection of the graphs demonstrates more subtle differences (e.g. "Dmt-depleted cells was significantly suppressed by depletion of Wapl (p5)" This is also particularly true for data on Figure 4A) The text should be modified to better match the data.

We went over the manuscript again and corrected the expression as it fits the result. In Figure 1F, although the statement in the main text was not changed, we now added a new result showing interphase cohesion by FISH, which clearly indicates that "cohesion defect in Dmt-depleted cells was significantly suppressed by depletion of Wapl"(p.6).

3. The localization of Dmt presented in figure 2 is very convincing and the microscopy stunning. However, the finding that cohesin does not follow the same localization, as previously described in neuroblasts, is quite puzzling. Could the authors speculate on why these differences may occurs?

As the referee pointed out, the cohesin localization in S2 cells seems to be different from the previous case in neuroblast cells (Oliveira et al., 2014). We confirmed the localization of Smc3 and Rad21 (Scc1) in both live cells and fixed cells and both showed uniform distribution in the S2 cell nucleus. In addition, we requested Rad21-EGFP cDNA from Dr. Raquel Oliveira and confirmed the Rad21-EGFP showed uniform localization in nucleus in living S2 cells (Appendix Figure S1C). Therefore, we presume that this difference may be result from different cell types. S2 cells are undifferentiated embryonic cells, which have more meiotic or early embryonic characteristics than in tissue-specific neuroblast cells. It would be intriguing possibility that Dmt itself or other heterochromatin factor recruits cohesin to heterochromatin in neuroblast cells and, as a result, heterochromatin-based cohesion establishment and the protection would be achieved as seen in S2 cells. We mentioned this in the Discussion section (p.16-17).

4. In figure 3A the authors show that upon Dmt RNAi, Scc1 is still present on chromatin, which is quite surprising considering published results and the authors own data. Are the levels the same? What about the levels of smc3 measured in fixed samples (as in Fig. 3B)?

It has been shown that vertebrate Sororin is dispensable for cohesin association with chromatin; this is the reason why Sororin is called "cohesion establishment factor" (Schmitz et al., 2007, Nishiyama et al., 2010). When Sororin is depleted in human cells, amount of chromatin-bound Scc1 is not apparently changed but it becomes more dynamic on chromatin, which could be only observed in FRAP experiment (Schmitz et al., 2007). In this sense, our result that Dmt RNAi did not decrease Scc1 amount on chromatin is consistent with the previous observations in vertebrates. Nevertheless, here we show the quantification of Scc1-mCherry intensity in pre-extracted Dmt RNAi cells (Figure for Referee 2, below). Dmt RNAi did not significantly change the amount of chromatin-bound Scc1.

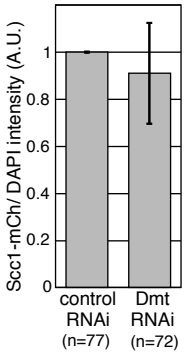


Figure for Referee 2

Scc1-mCh cells were treated with control or Dmt dsRNAs and Scc1-mCh intensities were measured in 77 and 72 cells in control and Dmt RNAi cells, respectively. Scc1-mCh intensity in controm RNAi cells was normalized to 1.

5. The authors convincingly demonstrate that Dmt binds to heterochromatin in a HP1-dependent manner. However, the claims regarding the importance of this interaction for cohesion should be toned down. In Figure 4 authors claim that WT Dmt rescues although looking at the graph it is clear that the rescue is only to about 50% and the mutants also rescue to about 30%. Although the tendency is there, the effects are rather mild (again, statistics could support the claims).

In the previous Figure 4, we intended to emphasize that the "over cohesion" phenotype seen in WT-over-expressing cells was significantly decreased in Δ6- or VEIE-expressing cells. Nevertheless, we changed the chromosome spread data to mitotic FISH results, where WT restored the cohesion to the similar extent to control RNAi cells (~70% of the mitotic cells), whereas VEIE restored cohesion in ~30% of the cells (new Figure 4A). We agree that VEIE could indeed partially restore the cohesion, presumably because of overexpression of VEIE protein, which could overcome the weakened function of VEIE mutant (new Figure 4B).

6. The results regarding PP2A interaction/localization are probably the strongest points of the paper. Although the rationale for the use of WAPL depletion is clear, Figure 6C should nevertheless include quantifications of the respective controls (wild-type cells) to clarify if the

bimodal behavior of wdb-GFP localization if also present in otherwise unperturbed cells, or a consequence of WAPl depletion (maybe WAPL itself also interacts with PP2A).

To quantify the Wdb-GFP intensity in control, Wapl, and Wapl+Dmt RNAi cells, we repeated the experiment. In the previous analysis, we included separated chromosomes, most of which were categorized as Wdb (-). As we shown in new Figure 6C, loss of cohesion (Scc1 RNAi) abolished Wdb accumulation on mitotic centromeres. Therefore, to directly evaluate the requirement of Dmt for Wdb localization, we measured Wdb-GFP and Dmt intensities only in cohered chromosomes in all conditions. In new Appendix Figure S5C-E, we calculated Wdb-GFP centromere/arm intensity ratios and plotted them against Dmt intensities. The results clearly showed that the reduction of Dmt signal intensities was correlated with the reduction of centromeric accumulation of Wdb-GFP. We mentioned this in the main text (p.12). In addition, as mentioned above, we added new Figure 6C to show that cohesin/cohesion is required for centromeric localization of Wdb.

7. For the FRAP data on figure 5GH, did the authors control for cell cycle stage of the cells analyzed? This should be important as cohesion stability if known to change significantly upon replication.

This is an important point that we did not mention. We can perform FRAP experiment only in S/G2 phase, because Dmt-GFP is degraded in G1 phase in a Cdh1-dependent manner (Figure EV1) and starts to be accumulated in S phase and reaches a maximum level in G2 phase. Therefore, if Dmt-GFP is present in the cells, it is a good indicator for S/G2 phase. We assumed that Dmt-ΔN116 is also degraded in the same kinetics as WT because degron(s) of Dmt was predicted to be present in its C-terminus (Figure EV1C). We added this explanation in the main text (p.11).

8. The rescue experiments presented in Figure 7 are indeed quite remarkable and a very convincing argument for the major claims of the manuscript. It would nevertheless be important to control that the levels of all constructs are equivalent (either WB or quantifications of GFP levels).

According to the suggestion, we added western blots for GFP proteins in new Figures 7D and 7F. Because we could not obtain good Sgo1 antibodies, we did not show the Sgo1 blots. However, as the Sgo1 siRNA used in this study has been already published in many previous

studies (McGuinness et al., 2005, Kueng et al 2006, Nishiyama et al 2010, and so on) and the Sgo1 RNAi phenotype was restored by expression of RNAi-resistant Sgo1, we are convincing that the Sgo1 knockdown did not show any the side effects.

Minor points:

9. The sentence "RNA interference (RNAi) of Dmt resulted in defective cohesion in the control cells (p5)" is confusing and should be rephrased.

We appreciate the correction. We rephrased the sentence.

10. Figure 1C needs a legend;

We confirmed that Figure 1C now has the legend.

11. The authors should included further details on how the data in figure 1D was scored? Are they measuring only G2 cells? How are they identified?

In all interphase FISH experiments, we considered that all the cells we analyzed were in S/G2 phase because a pair of FISH signal indicates that the genomic region has already replicated. We added this explanation in the main text (p.5-6).

12. Figure 1E lacks loading control (is the decrease in scc1 levels upon Dmt+Wapl RNAi consistent?)

We now repeated the experiment and added the tubulin blot loading control.

13. Figures 5F-G should include a label that it refers to Dmt-GFP

We now added the labels of Dmt full-GFP in Figure 5F and 5H.

In summary, this manuscript reports very exciting findings. Some of the conclusions need to be further supported, as the experimental set-up (multiple RNAi/rescue experiments) is prone to intrinsic experimental variability. However, if all the concerns are addressed and the conclusions are well documented this manuscript should be of prime interest for EMBO Journal readers.

2nd Editorial Decision 26 March 2017

Thank you for submitting your revised manuscript on Drosophila dalmatian to our editorial office. It has now been re-reviewed by the three original referees, and I am pleased to inform you that all of them are satisfied with the revision and supportive of publication. We shall therefore be happy to accept the manuscript for The EMBO Journal, pending the following minor editorial changes during a final round of minor revisions. In particular, I would appreciate if you went once more carefully through the results section and try to improve the presentation of the conclusions, as suggested by referee 1. It would also be helpful if you could add a schematic summary figure, which could in addition serve as a basis for the synopsis figure that will accompany the bullet points that you already provided.

REFEREE REPORTS

Referee #1:

The authors have greatly improved their manuscript with the addition of many new experiments that strengthen and extend the conclusions of the original version. I appreciate the inclusion of quantification of FISH experiments and the experiments in the appendix explaining the identification of binding motifs. It is a very large body of work and moves the field forward a great deal. The overall findings, that Dmt performs the function of shugoshin and sororin in the fly are very exciting and will be of broad interest.

My only comment for improvement is that the manuscript is quite hard to follow in places, particularly the sections dealing with Dmt localization dependency on heterochromatin and cohesion. It should be emphasised in each section whether the conclusions refer to interphase or mitosis, as the dependency on cohesion seems to change in this two stages, but this is not especially pointed out. I suggest that the authors go through the manuscript and ensure conclusions of each experiment are stated clearly. Perhaps a schematic summary figure would help make the roles and localization dependencies clear at each stage too. For readers outside the field, the main points may get lost in the details as the paper stands.

Referee #2:

The authors properly addressed all my concerns. The story is very exciting and should be published immediately.

Referee #3:

This revised version of the manuscript, by Yamada and co-workers, addresses most of my previous concerns in a satisfactory manner. In particular, 1) the new FISH experiments added for the quantitative evaluation of sister chromatid cohesion is a clear improvement over the arbitrary "cohesed" vs "non cohesed" 2) the reproducibility of the experiments was clarified, 3) MG132 experiments backing up the FISH strongly strengthen the conclusions; 4) All the loading controls that were missing were added 5) all other issues were either directly addressed in the manuscript or the authors provided a satisfactory answer. I can therefore fully recommend its publication.

2nd Revision - authors' response 04 April 2017

I would like to thank the referee #1 for the suggestions to improve our manuscript. According to the suggestions, we 1) emphasized that HP1-dependent Dmt localization in interphase is converted to cohesin-dependent localization in mitosis in the result section, 2) added new schematic figure depicting the exchange and dependencies of localization and cohesion (new Appendix Fig S8), and 3) went through the text and clarified the descriptions.

3rd Editorial Decision 06 April 2017

Thank you for submitting your final revised manuscript for our consideration. I am pleased to inform you that we have now accepted it for publication in The EMBO Journal.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Tomoko Nishiyama
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2016-95607R

Reporting Checklist For Life Sciences Articles (Rev. July 2015)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	In mitotic FISH to evaluate mitotic cohesion, number of FISH signals were counted in more than 20 cells in each condition and the experiments were repeated at least 3 times. In interphase FISH to evaluate cohesion establishment, distances between 2 FISH signals were measured in more than 30 cells in each condition.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Samples were not excluded from analysis, except in special cases e.g. bad image quality.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	NA
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Statistical tests were justified as appropriate and clearly understandable.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Normality was tested by D'Agostino & Pearson omnibus normality test.
Is there an estimate of variation within each group of data?	Yes
Is the variance similar between the groups that are being statistically compared?	Multiple comparison in Figure 6H, variance was evaluated with Browne-Forsythe test and Bartlett's test and the variances were similar between the groups. All the other single comparisons were performed with nonparametric Mann-Whitney U test.

C- Reagents

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6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Described in the materials and methods section.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

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D- Animal Models

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10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

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12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	NA
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	We included all dataset in Extended View 1-4, Appendix Figure S1-7
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section. Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	This section was not included in the manuscript.
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

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